



APOE4, oxidative stress and decreased repair capacity - a no-brainer. Faulty lipid metabolism and increased levels of oxidative damage may be risk factors in the pathogenesis of late-onset dementia

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Abstract

Dementia is very common in the elderly and its incidence increases in an age-dependent fashion. Alzheimer's disease and vascular cognitive decline are the most common cases of dementia in the elderly. Amyloid burden and increased levels of oxidative damage have been implicated to play significant roles in the pathogenesis of late-onset dementia. In this paper we propose that there are three major genetic factors that may modulate the risk for dementia in later life: carriership of *APOE* variant alleles, carriership of mitochondrial DNA of haplogroups associated with ineffective oxygen utilisation (specifically, haplogroup H) and carriership of genetic polymorphisms conferring subtly deficient DNA repair. All three factors are quite common in the European populations. Each of these three factors may not have significant effect on the phenotype when taken separately, but when combined in the same genotype, the effects may be cumulative. Further studies are needed in order to elucidate the genotype-phenotype relationships and provide a reliable basis for assessment of the genetic risk for sporadic late-onset dementia. Lifestyle alterations and therapies targeted at decreasing the oxidative burden to aging cells and tissues may decrease the risk for neurological decline in later life.

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Abbreviations: AD – Alzheimer's disease, APOE – apolipoprotein E, ATP – adenosine triphosphate, BER – base excision repair, CAA – cerebral amyloid angiopathy, CNS – central nervous system, FTD – frontotemporal dementia, HD – Huntington's disease, IMT – intima-media thickness, IRC – individual repair capacity, MCI – mild cognitive impairment, MND – motor neuron disease, NER – nucleotide excision repair, NMDA – N-methyl-D-aspartate, PD – Parkinson's disease, PDAPP – PDGF promoter expressing amyloid precursor protein, ROS – reactive oxygen species, SOD – superoxide dismutase, VCI – vascular cognitive decline.

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1. Dementia with late onset - many causes, (potentially) one disease

Dementia is one of the top causes for of disability and dependency after the 6th decade of life and the fourth most common cause of death in developed countries. The most common cause of dementia in the elderly is Alzheimer's

disease (AD), followed by vascular dementia (VD). The latter is also commonly referred to as vascular cognitive impairment (VCI), a rather unspecific term covering moderate and severe cognitive decline developing after

stroke/s and small-vessel disease. The prevalence of AD in those aged 60-65 is less than 1 %. It increases steadily up to the age of 85, when it may reach > 35 % of the elderly [1]. The prevalence of VCI is 4-5 % in individuals over 65 years of age and 10-20 % of all dementias [2]. The prevalence of dementia decreases after the age of 85 to values below those seen in 'younger old' [3]. VCI and AD may coincide in the same patient (mixed dementia). It is believed that about 50 % of all dementias are, in fact, mixed dementias [4]. Other diseases and conditions with onset in advanced age such as Parkinson's disease (PD) and frontotemporal dementia (FTD) may also contribute to the prevalence of dementia in the elderly. Assessment of the risk for dementia in later life is, at present, unreliable, except in familial early-onset cases of AD. The single most important risk factor for development of dementia is advancing age, but even among the 'oldest old' (>85 years of age) there are no less than 50 % of individuals showing no signs and symptoms of dementia [5,6]. Female sex, history of stroke/s and presence of mild cognitive impairment (MCI) generally increase the risk for dementia.

Much effort has been put into identification of the genetic and environmental factors that contribute to the development of late-onset dementia. No environmental factor increasing significantly the risk for the development of AD and Parkinson disease with dementia has been identified, except history of head trauma [7, 8]. Less than 7 years of formal schooling has also been reported to be a risk factor for AD in later life [9], although this may be related to initial cognitive reserve. AD and PD are known to run in families, but the majority of the cases (up to 90 %) are sporadic. More than 10 genetic loci have been linked to increased risk for development of AD, but significant association (allowing for reliable estimation of the risk for AD) has generally been identified for the familial early-onset (before the age of 65) forms only [10]. Significant association with the risk for development of AD has been shown for the human genes *APP* (coding for amyloid beta A4 precursor protein) and *PS1/PS2* (coding for catalytic subunits of gamma-secretase responsible for proteolytic cleavage of the amyloid precursor protein in the amyloidogenic pathway) [11-13]. Carriership of mutations in these genes is usually associated with significantly increased lifelong risk (over 90 %) for early-onset (before 65 years of age) AD. Genetic variants conferring susceptibility to late-onset AD have also been described (in the gene coding for APP-binding protein B2 (*APBB2*); the gene coding for endothelial nitric oxide synthase 3 (*NOS3*), and others), but their penetrance is generally incomplete [14, 15]. At present, there is no treatment that may delay or prevent the development of AD. Females have been reported to be at higher risk for developing AD than males [16, 17] suggesting that the

levels of sex hormones may play a role in the pathogenesis of AD.

Unlike AD, vascular cognitive decline is considered potentially preventable as there are defined measures that may decrease the risk for vascular disease and stroke (maintenance of blood glucose and cholesterol within reference ranges, maintenance of arterial pressure at or below 120/80, body weight adequate for age and sex, giving up harmful habits such as smoking, etc.). It is, however, difficult to predict who would develop vascular dementia in a cohort of at-risk individuals (or even among post-stroke patients) in order to select those that may benefit from additional therapy in order to prevent development of dementia. Tomlinson suggested that the development of cognitive decline was likely to occur in post-stroke patients in which the total infarcted volume was ≥ 100 ml [18]. Later it was suggested that not only the total lesion volume but also the location of the lesion/s were important factors in the establishment of the risk for post-stroke cognitive decline [19]. The assessment of the risk for VCI after stroke is further complicated by the fact that some cell populations are exquisitely vulnerable to ischemic insults and may succumb to cell death even in the absence of focal ischemic lesions in their immediate vicinity; or after global ischemia [20]. Apparently, factors other than the direct effects of brain ischemia or haemorrhage may increase neuronal vulnerability after stroke. Similarly to AD, females have been reported to be at slightly higher risk for VCI than males, even though the risk for vascular incidents is considered to be higher in males than in females [21].

There is one notable genotype-phenotype correlation that has been demonstrated to be valid for both AD and VCI - namely, the association between carriership of the epsilon4 (*APOE4*) allele of the apolipoprotein E gene (*APOE*) and the increased risk for dementia. Evidence supporting the role of carriership of *APOE4* in the risk for AD has been accumulating for years, but the association has, until recently, remained purely phenomenological. Carriership of *APOE4* has been shown to be at increased risk for both early-onset and late-onset AD [22]. Apparently, the risk-modulating properties of E4 function on genetic as well as epigenetic level. In later years, an association between carriership of *APOE4* and the risk for VCI has also been demonstrated [23, 24].

Oxidative stress has been implicated in physiological as well as pathological brain aging as a major factor triggering and promoting neuronal death. Oxidative damage has been proposed as a major pathogenetic mechanism in tissue damage (including vascular and neuronal damage) in diabetes type 2 [25]. Evidence of oxidative damage has been found in proteins, lipids, and DNA from autopsied brains and peripheral

tissues of patients with AD and PD [26]. Recently, a theory that late-onset neurodegenerative diseases had a common pathogenetic mechanism based largely on premature neuronal death triggered and promoted by unrepaired oxidative damage has been developed [27]. Oxidative stress has been strongly suspected as the main culprit for neuronal loss in ischemic strokes, brain microhaemorrhages and head trauma [28-30]. Oxidative phosphorylation is the main source of oxidative stress in living cells. Multiple polymorphic changes of mitochondrial DNA (usually clustered together on the same DNA molecule and thereby referred to as haplogroup) conferring lower-or higher-than-average oxygen consumption and, respectively, lower-or higher-than-average levels of production of ROS have been described [31]. Specific mitochondrial haplogroups are more commonly seen in individuals representing the pattern of ‘successful aging’ - that is, preservation of mental and physical capacity well into advanced age, whereas other haplogroups may be associated with increased risk for late-onset disease, including neurodegenerative disease and vascular disease [reviewed in 32]. One may hypothesise that in cases where the mitochondrial metabolism conferred by carriership of specific polymorphisms generates higher-than-average output of ATP and, respectively, higher amounts of ROS, the cell may enjoy the benefits of increased rates of ATP production in the short term (in young and healthy individuals) but may suffer from the oxidative burden resulting from the additional oxidative damage in the long term (in advanced age).

Eukaryotic cells manage a high daily amount of genotoxic damage ($\geq 10^4$ events/day) [33]. Normal cells employ a variety of physiological mechanisms checking and repairing genomic damage or initiating programmed cell death in cells that have sustained too much damage. These mechanisms manage genotoxic damage fairly efficiently in young age. Even among young and clinically healthy individuals, however, there are differences (sometimes - significant differences) in the capacity to handle everyday genotoxic damage. These differences tend to become more pronounced in advanced age and/or in conditions of increased genotoxic stress (due to endogenous factors - e.g. ischemia, or to exogenous factors - physical or chemical genotoxic agents, including genotoxic treatments, etc.). The individual capacity to identify and repair genomic damage and the associated capacity to assess damage and make decisions whether to rescue and repair damaged cells or trigger their self-destruction by apoptosis (management of genomic integrity) is presently referred to as ‘individual repair capacity’ (IRC). IRC is a function of the genetic background of the individual (carriership of genetic variants conferring subtly decreased or, more rarely,

subtly increased capacity to handle genotoxic damage); the individual’s current general status (young or aged, healthy or affected by specific diseases and conditions) and the environment (levels of common genotoxic factors, additional specific factors). It could be expected that the impact of everyday genotoxic factors (e.g. oxidative damage) may be augmented in individuals with genetic (and, therefore, lifelong) lower-than-normal capacity to identify and repair genotoxic damage.

In this paper we propose that the genetic risk for late-onset sporadic dementia may be determined by three major factors: carriership of common *APOE* variants other than E3 (predominantly E4 for AD, E4 as well as E2 for vascular cognitive decline); carriership of mitochondrial DNA of haplotypes associated with generation of higher-than-average levels of ROS; and subtly decreased capacity for identification and repair of genotoxic damage. Each component has its individual contribution in the risk for dementia (although their combination in the same genotype may have cumulative effect) and environmental factors may modify this risk. Knowledge about the physiologic and pathologic mechanisms behind of these factors may, potentially, be used as a basis for development of strategies for early intervention in order to decrease the risk or delay the onset of dementia in later age. Information about the inter-individual variance in carriership of alleles associated with impaired lipid profile and/or increased risk for amyloid neuropathology; inefficient mitochondrial energy management and/or decreased capacity for repair of genotoxic damage may assist in the construction of a panel for assessment of the risk of late-onset sporadic dementia and may be used as a basis for informed lifestyle choices in at-risk individuals in order to decrease genetic risks for late-onset dementia.

2. Amyloid deposition in the brain - what, where and how.

Two major types of pathological structures are commonly observed in autopsied brains of patients with dementia - amyloid beta-peptide aggregates (amyloid plaques) and tau protein depositions (neurofibrillary tangles). Amyloid plaques and neurofibrillary tangles were described by Dr. Alois Alzheimer in 1907 and have since been considered hallmarks of AD. Amyloid-beta deposits (predominantly 42 amino acid residues long, Abeta42) may be observed in the brain parenchyma (amyloid plaques) and/or in the walls of leptomeningeal and cortical brain vessels (cerebral amyloid angiopathy (CAA) due to deposition of amyloid-beta peptide 40 amino acid residues long, Abeta40) [34]. CAA is associated with significantly increased risk for massive lobar haemorrhage (haemorrhagic stroke) and for serial microhaemorrhages producing transient neurologic

symptoms and/or progressive neurological deterioration. The degree of involvement of brain blood vessels in CAA may vary (from mild to severe) and the type of brain vessels involved may also be different (involving brain arteries, arterioles, veins and venules - CAA2 and capillary as well as larger-vessel - CAA1) [35]. Capillary amyloid deposits in CAA1 tend to infiltrate the surrounding parenchyma and promote the formation of parenchymal amyloid plaques typical of AD [36]. CAA (specifically, CAA1) is almost always present (80-90 %) in brains of patients with AD.

Amyloid deposition in the vessel wall is initially confined to the outer basement membrane. In later stages of CAA, the smooth muscle layer in the vessel wall is almost completely obliterated and only the endothelial layer is relatively spared [37]. It is believed that the amyloid beta aggregates have a direct toxic effect on the smooth muscle cells and the endothelial cells in the vascular wall, accelerating their death and increasing the risk for breach of the integrity of the vessel wall. Different types of cerebral amyloid angiopathy are believed to increase the risk for different types of dementia via different mechanisms (CAA1 - by increased parenchymal amyloid deposition, increasing the risk for AD; CAA2 - by increased risk for cerebral haemorrhages). CAA-associated vasculopathy is a major risk factor for massive intracerebral haemorrhage as well as for microhaemorrhages [reviewed in 38]. The latter may increase the risk for future larger haemorrhages and for cortical superficial siderosis that is also associated with increased risk for development of dementia [39]. Cerebral microhaemorrhages may occur in brains unaffected by amyloid neuropathology, but the pattern of their distribution is different in patients with high amyloid burden and in patients with another significant risk factor for stroke, namely, hypertension [40]. The presence of CAA alone may be associated with increased risk for dementia, as pre-existing dementia was reported to be common in patients with brain haemorrhages due to extensive CAA [41]. The authors of the cited study proposed a dual mechanism of pathogenesis of dementia in individuals with extensive CAA involvement. On the one hand, there was high likelihood that a patient with amyloid vascular pathology would also have parenchymal involvement, associated with increased risk for AD. On the other hand, the presence of CAA was likely to cause a large intracerebral haemorrhage and/or serial microhaemorrhages [41]. The relationship between intracerebral haemorrhage and dementia apparently works both ways, as newly diagnosed dementia is a significant risk factor for intracerebral haemorrhage [42].

Brain ischemia increases the risk for dementia, although indirectly. Ischemic insults to the brain were shown not to have a significant short- and medium-term effect on the cognitive status but increased the risk for

development of AD-type dementia later [43]. Unlike CAA, where small-vessel involvement is associated with increased risk for AD, the presence of large-vessel (but not small-vessel) atherosclerotic cerebrovascular disease is strongly associated with development of parenchymal amyloid plaques [44]. Local impaired perfusion and hypoxia due to the presence of atherosclerotic plaque and the associated changes in the endothelial wall of brain vessels are believed to enhance the production of amyloid-beta peptide. The resulting amyloid angiopathy, in turn, perpetuates the hypoperfusion and the oxidative stress in the vessel wall, increasing the risk for endothelial breach [45, 46].

Brain amyloidosis is undoubtedly associated with increased risk for dementia, but it is still unclear whether amyloid is a driving factor in the pathogenesis of dementia or an outward manifestation of another pathological process. Amyloid neuropathology is very common in patients of dementia of either vascular or AD origin, but is not uncommon in nondemented aged brains too. In studies in living nondemented patients or patients with MCI aged 80-85, evidence of amyloid deposits was found in 55 % of the nondemented individuals and 68 % of those with MCI [47]. Recently, was shown that in individuals aged 50 years, about 10 % of those assessed as having normal cognition and 27 % of these with mild cognitive decline had evidence of beta-amyloid neuropathology [48]. The same study showed that in individuals aged > 80, the prevalence of amyloid neuropathology was > 40 % for those with intact cognition and about 70 % of these diagnosed with MCI [48]. The authors concluded that presence of cerebral beta-amyloid deposits was age-dependent and preceded the onset of cognitive decline by 20-30 years, but it could not be identified as an etiological factor for dementia. It is unclear whether, had these 'asymptomatic' subjects lived, they would have, at some point, progressed to overt dementia. Apparently, amyloid in the brain is commonly seen in those with dementia, but not all individuals with amyloid neuropathology progress to dementia. This has prompted some authors to propose that the presence of amyloid in aged brains may not be the result of a disease but, rather, an accompanying effect on another, yet unknown process, or even a compensatory mechanism that eventually failed (in individuals that received the diagnosis of dementia prior to their death) or still held strong (in those that remained asymptomatic) [49].

The prevalence of brain amyloidosis and the degree of involvement is significantly higher in individuals carrying at least one copy of a specific genetic variant - namely, the E4 allele of the apolipoprotein E (*APOE*) gene. In the study of Mathis et al. [47] conducted in living nondemented people aged 80-85 years, carriership of the *APOE4* allele was 5 times as prevalent in study subjects with confirmed beta-amyloid brain pathology (30 %) than

in those without evidence for beta-amyloid deposition (6%). Apparently, carriership of *APOE4* increased the risk for amyloid beta pathology. The latter, in turn, may increase the risk for dementia, although this risk may be modulated by other factors.

3. APOE - a simple lipoprotein transporter, a major amyloid peptide metabolism modulator or in-between?

Apolipoprotein E gene codes for a protein involved in the binding, transport and clearance of lipids, lipid-soluble vitamins and cholesterol [50]. It is actively expressed in many tissues, including the liver, the adipose tissue, the walls of major arterial vessels and the brain. There are six isoforms of APOE, coded by six different alleles, of which three are common (epsilon (E)2, E3 and E4) in virtually all populations and the other three (E1, E5 and E7) are quite rare and may be population-specific [51]. E2, E3 and E4 alleles differ by single nucleotides in two specific sites producing substitutions of Cys with Arg at amino acid residues 112 and/or 158 (E2-Cys/Cys, E3 (Cys/Arg), and E4 Arg/Arg) [51]. The most common *APOE* allele - E3 has not been associated with serious phenotype modulating effects and is usually considered wildtype. Carriership of the other two common alleles of *APOE* may be associated with impaired clearance of lipoprotein particles, resulting in increased levels of plasma cholesterol and/or triglycerides. Carriership of *APOE4* allele is associated with increased total and LDL cholesterol and triglycerides in plasma (a 'hyper-lipemic' lipid profile, hyperlipoproteinemia type III), and confers increased risk for vascular disease [52-54]. Carriership of a single E2 allele of *APOE* may be associated with lower cholesterol levels than E3 but, at the same time, higher triglyceride levels than E3 [55]. Carriership of a single E2 allele has been reported by some authors to be associated with a 'normo-lipemic' lipid profile, conferring a low-grade protection from vascular disease [54, 56, 57]. E2-allele containing genotypes have been associated with lowest intima-media thickness (IMT, a crucially important parameter in assessment of accumulation of atherosclerotic plaque) and, respectively, with lowest degree of atherosclerotic involvement of the vascular wall of the three common *APOE* variants. The IMT values in *APOE3* carriers were higher than IMT in E2 allele carriers and E4 genotypes were associated with highest IMT and extensive atherosclerotic involvement [58]. In its homozygous state, however, E2 allele may, similarly to E4, be associated with increased risk for hyperlipoproteinemia type III [52, 59].

APOE4 has been known for decades as a powerful genetic factor increasing the risk for late-onset dementia. In carriers of a single *APOE4* allele, the risk for development

of AD and VCI is significantly higher than the population risk (1.5-2 fold for VCI, 3-4-fold for AD) and becomes very high in homozygous carriers of two *APOE4* alleles (3-4-fold for VCI; 7-8-fold for AD) [22, 23]. The strength of the association may be modulated by other factors such as ethnicity, biological sex and, possibly, other genetic factors. The association between E4 carriership and the risk for AD might be stronger for patients of Asian ethnic origin than for patients of Caucasian origin, as the prevalence of E4 was shown to be lower in individuals of Asian ethnic origin than in Caucasians but the prevalence of AD was similar among the two studied populations [55]. The risk for AD conferred by carriership of a single *APOE4* allele was shown to be insignificant for males and significant for females [17, 22]. Carriership of a single E2 allele has been shown to have a mild protective effect against AD in some populations and was associated with milder AD phenotype [16, 60].

Hippocampal atrophy may be among the earliest findings in AD, developing years and decades before dementia may become manifest. The rate of loss of neurons from the hippocampus and the amygdala in AD was reported to be dependent on the *APOE4* genotype [61,62]. A study published in 2011 reported that the hippocampal volume in young and healthy carriers of *APOE4* was already significantly smaller than in controls with genotypes containing no E4 alleles [63].

Carriership of E2 or E4 *APOE* alleles was reported to be associated with more severe vascular amyloid pathology [64]. *APOE4* is a risk factor for development of CAA, especially CAA1 (capillary type) [65]. The prevalence of *APOE4* allele was estimated to be > 4 times higher in brains affected by the CAA1 than in brains with CAA2 and controls. At the same time, in brains affected by CAA2, the prevalence of *APOE2* alleles was higher than in brains with CAA1 and control brains [35, 65]. This may explain the at least partly the association between *APOE4* and the increased risk for both early- and late-onset AD - as capillary CAA was associated with increased risk for parenchymal involvement typical of AD, it was an independent risk factor that would work in virtually all types of genetic backgrounds. The risk for AD in E2 carriers may be lower probably because of the fact that E2 allele is rarely associated with capillary CAA.

Carriership of *APOE4* increases the risk for post-stroke cognitive decline [23], but this might be an indirect effect, as *APOE4* is also associated with increased risk for ischemic stroke [54,66]. An inverse correlation between has been reported between carriership of a single allele of *APOE2* and the risk for ischemic stroke [67]. Carriership of the E2 variant alleles are, nevertheless, at increased risk for lobar intracerebral haemorrhages as well as microhaemorrhages [68]. Carriers of the relatively rare E2/E4 genotype are at significantly increased risk for recurrence of intracerebral

haemorrhage [69]. The homozygous E2/E2 genotype is associated with increased risk for early-onset vascular disease/cerebral haemorrhage, probably because of the impaired lipid metabolism conferred by *APOE2* and the increased risk for cerebral haemorrhage in the presence of CAA [67].

APOE4 has been shown to modulate directly the metabolism of amyloid precursor protein in the amyloidogenic pathway. One of the short-term effects of head trauma is transiently increased metabolism of the amyloid precursor protein, possibly stimulating neurite outgrowth and cell survival [70]. In transgenic mice expressing human *APOE4*, head trauma resulted in lowering of the levels of amyloid precursor protein and the amyloid beta peptide in the hippocampus whereas the levels in *APOE3*-expressing transgenic mice, wildtype mice and *APOE*-deficient mice were not affected. Amyloid deposition is one of the common late sequelae of head trauma and head injury is a recognised risk factor for the development of dementia later in life. In brains of young (9-10 months) transgenic mice overexpressing human amyloid beta peptide (PPDAPP mice) and *APOE3* or *APOE4* and subjected to traumatic brain injury, different amounts of amyloid accumulated in the hippocampus after brain injury - 20 % for *APOE3*-expressing and 56 % for *APOE4*-expressing PDAPP mice [71].

At present, carriership of *APOE* variants is considered an independent risk factor for dementia in later life that may be augmented by other factors in the genetic background of susceptible individuals as well as environmental factors. The risks for dementia ('true' AD or cognitive decline after intracerebral haemorrhage/s and ischemic stroke/s) due to carriership of *APOE4* are partly associated with modulation of the metabolism of APP that, in turn, increases the risk for parenchymal and/or vascular deposition of amyloid beta peptide, and, probably, partly associated with increased risk for atherosclerotic vascular disease because of the modulating effects of the *APOE* variant alleles on the lipid metabolism. It is still a subject of debate whether carriership of *APOE* variants has direct effect on neuronal loss in dementia. Beta-amyloid has been found to directly upregulate the expression of caspase-3 (a major executor caspase) in vitro [72, 73]. Carrier status of *APOE2* and *E4* variants in mice was reported to be directly associated with the rates of neuronal apoptosis rates after mechanical neuronal injury [74]. Specifically, transgenic mice expressing human *APOE4* had higher intracellular calcium levels and, respectively, higher apoptosis rates after neuronal injury than mice expressing human *APOE3* and control mice [74]. Whether this pertains to mammals other than rodents and, specifically, to humans, and whether the putative effect of *APOE* on neuronal death also works under conditions other than traumatic brain injury is still unknown.

4. Neuronal cell death in dementia - it breaks where it's thin?

Mass cell death affecting specific neuronal populations in the grain or more generalised brain atrophy is almost universal post-mortem finding in brains of patients with dementia. Mild neuronal loss is normal in the course of brain development and in brain aging, but the rates of neuronal loss and the affected areas of the brain may be different in physiological and pathological brain aging. For example, slowly progressing hippocampal atrophy is commonly seen in aging brains [75]. Much faster hippocampal atrophy, however, is a hallmark of very early AD [76, 77].

Neuronal loss in dementia is rarely diffuse (except, perhaps, in the very advanced stages) but, rather, follows a pattern that may be different depending on the type of the underlying condition. In focal cerebral ischemia, the severe impairment in oxidative phosphorylation and the resultant oxidative stress at the lesion site causes mass cell death by necrosis or apoptosis in a matter of minutes to hours after the ischemic incident. In the penumbra of the ischemic lesion oxidative phosphorylation is impaired due to hypoperfusion and hypoxia and the levels of oxidative stress are significantly higher than normal; but neural tissue in these regions may survive, especially when supportive measures to improve brain perfusion have been instigated in the immediate acute period. Even with active treatment, however, many more neurons (including neurons in locations relatively distant from the lesion site) may succumb to cell death days to months after the incident. This delayed cell death may affect the potentially viable neurons in the penumbra of the stroke as well as the neuronal populations elsewhere in the brain (especially in the hippocampus, the basal ganglia, and the Purkinje cells in the cerebellum) [20]. The latter may die even in the absence of ischemic or hemorrhagic insults in their vicinity. The neuronal loss seen in AD brains is more generalised than in other neurodegenerative diseases such as Parkinson's disease, Huntington's disease (HD) and motor neuron disease (MND) where mass cell death affects specific neuronal populations) but still, there are areas that are affected more severely than others and their involvement begins early in the course of the disease. Such are the hippocampus and its connected structures (e.g. the entorhinal cortex) - very early, usually at pre-symptomatic level; and, later, the posterior cingulate cortex and the amygdala [61, 77, 78]. The percentage of lost neurons was shown to differ by as much as 30 % between normal aged controls and patients with AD brains [76]. The rates of neuronal loss from different hippocampal subdivisions were also different between AD brains and normal aged brains [77]. Some of the vulnerable neuronal populations may overlap in vascular cognitive decline and AD.

Hippocampal neurons may succumb to cell death after a rapid acute increase in the levels of oxidative stress (ischemic incidents, global ischemia) as well as secondary to long-term chronic oxidative stress (neurodegenerative disease). Hippocampal CA1 and CA2 subfield neurons are commonly affected in post-stroke cognitive decline as well as in Alzheimer disease and mixed dementia [76, 79]. Elevated total and LDL cholesterol levels, diabetes type 2 and smoking (all of them major risk factors for vascular disease and stroke) were shown to be associated with smaller hippocampal volume and smaller neural volume in the entorhinal cortex in nondemented elderly males [80].

The causes for increased susceptibility of some (but not all) neurons in the adult CNS after stroke and in neurodegenerative disease are still elusive. Toxic over-stimulation of the receptors for excitatory amino acids (glutamate, NMDA and others) in neuronal cells (excitotoxicity) has been implicated in the pathogenesis of dementia after acute brain trauma, stroke and in neurodegenerative disease [81]. NMDA receptors (synaptic and extrasynaptic) are present in the majority of adult CNS neurons. Activation of the synaptic receptors stimulates pro-survival signalling in neurons whereas activation of extrasynaptic NMDA receptors results in unwarranted opening of the channel and rapid influx of Ca^{2+} cations into the cell, triggering cell death by necrosis or apoptosis via the endogenous mechanism [82]. The presence of amyloid beta peptide depositions has been putatively associated with localised induction of release of glutamate from the neighbouring astrocytes and dysregulation of the synaptic/extrasynaptic NMDAR balance, eventually causing predominant activation of the extrasynaptic NMDAR receptors and, eventually, cell death [83]. Mass neuronal death in neurodegenerative disease may be accompanied by stimulation of the CNS neural progenitor niches and increased levels of neurogenesis [84].

Adult CNS neurons are extremely long-lived (potentially, as long as long the organism lives) and are rarely, if ever, replaced. Thus, neurons are subjected to chronic, high-level genotoxic damage (predominantly oxidative damage caused by ROS generated in the course of normal cell metabolism). The capacity to detect and promptly repair genotoxic damage is essential for neuronal survival and the maintenance of their functional capacity. As age advances, the enzymatic activities functioning in the detoxification of oxidised substrates such as superoxide dismutase (SOD), catalase, glutathione transferase, and others gradually decreases. The efficiency of the cellular machinery recognising and repairing genotoxic damage may also decline over time. As a result, the levels of oxidative stress generally increase with age, thereby increasing the risk for accumulation of unrepaired

damage. The rates of occurrence of 8-oxoguanine, 8-hydroxydeoxyguanosine and double-strand breaks increase in an age-dependent manner in virtually all types of mammalian cells, including CNS neurons [85, 86]. Eventually, neurons that have been damaged beyond repair may be lost to apoptosis. The rate of physiological loss of neurons, however, is not as nearly as high as the rate seen in neurodegenerative disease. At present, it is believed that the cause for the mass cell death observed in neurodegenerative disease may be abnormal triggering of the programmed cell death mechanism caused by accumulation of oxidative DNA damage [87, 88].

5. Breath of life (and death) - increased ATP production is associated with increased oxidative stress

Virtually all cells in multicellular bodies are dependent on oxidative phosphorylation for production of ATP, but neurons are specifically vulnerable to oxygen and ATP deficiency because of their high metabolic rate. Neurons have high mitochondrial content with uneven intracellular distribution and the individual mitochondria are highly mobile within the neuron in order to comply with the requirements for ATP production in different neuronal segments [89]. Oxidative phosphorylation is unavoidably accompanied by generation of reactive oxygen species (ROS) - peroxide and superoxide anions, hydroxyl radicals, singlet oxygen and others. ROS are usually promptly metabolised (e.g. superoxides converted by designated enzymatic activities to oxygen and peroxide (the latter being eliminated by catalase) or scavenged (e.g. hydroxyl radicals - by endogenous free radical scavengers such as glutathione or exogenous scavengers such as tocopherol and resveratrol) [90]. ROS are short-lived, generally unstable and highly reactive molecular species that may react with virtually all kind of biological molecules; therefore, there is always risk for oxidative damage of cellular proteins, lipids and DNA. The level of oxidative damage in living cells depends on the endogenous production of ROS and the capacity to handle oxidative damage. There may be many causes for increased levels of ROS in living cells - impairment in cell metabolism (hyperglycemia, hyperlipidemia); decreased availability of glucose and oxygen (e.g. localised or generalised hypoperfusion due to vascular disease); carriership of genetic variants of mitochondrial DNA associated with higher-than-average generation of ROS; or ineffective mitochondrial metabolism. The latter may be inherited (associated with carriership of mutations in mitochondrial DNA resulting in inefficient electron transfer (mitochondrial disease) or may occur on a somatic level in later age. Neurons carrying mitochondria with de novo deletions of mitochondrial DNA associated

with deficiencies in the electron transport chain were found in brains of individuals with PD and in normal aged individuals, with statistically significant difference between the prevalence of deletions between the two groups [91]. Similarly, deletions in mitochondrial genes resulting in cytochrome c oxidase (COX) deficiency were found in hippocampal neurons of nondemented elderly individuals as well as in brains of age-matched patients with sporadic AD [92]. The prevalence of the deletions in AD brains was significantly higher than in nondemented controls.

The topological site of production of ROS is the inner mitochondrial membrane; therefore, it is the mitochondrial proteins, lipids and DNA that generally bear the brunt of oxidative damage. There are several features in the higher-level organisation of mitochondrial DNA that make it specifically vulnerable to oxidative damage. Among these are the absence of histone packaging and the high gene density of mitochondrial DNA. There are very few regions in mitochondrial DNA where an alteration in DNA sequence or structure would not result in a severe pathological phenotype. The sites in mitochondrial DNA where benign heritable changes in DNA sequence may occur are clustered predominantly in the control region where the origin for replication of the leading strand and the promoters of major genes coded by mitochondrial DNA are located. Several polymorphisms occurring within the same DNA molecule are commonly referred to with the term haplogroup. More than 15 currently existing haplogroups of mitochondrial DNA have been described [93, 94]. Different mitochondrial haplogroups may be associated with varying efficiency of oxygen utilisation [95]. Generally, the higher the oxygen consumption, the higher amount of ATP synthesised, and, respectively, the higher the levels of ROS and the daily amount of oxidative damage to DNA. Haplogroup H is associated with highest oxygen consumption of all haplogroups and, respectively, highest output of ATP and ROS [31,96]. It is also the most common haplogroup in European populations. Carriership of mitochondrial DNA of haplogroup H has been associated with increased risk for several degenerative diseases such as osteoarthritis [97] and hypertrophic cardiomyopathy [98]. In carriers of haplogroup H the risk for some of the complications of diabetes type 2 (diabetic retinopathy and neuropathy) is significantly increased [99]. Increased prevalence of mitochondrial haplogroups H and T has been noted in individuals affected with late-onset sporadic AD [100-102]. Specifically, sub-haplogroup H5 (common in Southeast Europe) was associated with > 2-fold increased risk for AD in European populations independently of *APOE4* status [101]. Concomitant carriership of haplogroup H is associated with earlier onset of

neurological symptoms in carriers of the CAG repeat that is expanded in Huntington's disease [103]. Carriers of mitochondrial DNA of haplogroup H may also be at increased risk of sporadic PD [104]. Apparently, the most common European haplogroup is associated with increased risk for several serious late-onset diseases, that is, it is clearly a disadvantage at late age. Haplogroup H and its ancestral haplogroup R (common in Asian populations), nevertheless, is associated with significantly increased chance for its carriers to survive periods of caloric restriction [105] and superior rates of survival after severe sepsis and septic encephalopathy [106]. Thus, haplogroup H may have been selected for in the course of the human evolution as it conferred to its carriers a selective advantage that may have improved their chances to survive childhood, reach sexual maturity and, respectively, contribute amply to the genetic pool of the population. Since the average human lifespan exceeded 45 years only in the last 100 years, the late consequences of carriership of haplogroup H such as increased risk for development of neurodegenerative disease and, possibly, other diseases and conditions in advanced age may have become apparent only recently. This may represent an example of 'antagonistic pleiotropy', a phenomenon in which certain genetic traits may put their carriers at selective advantage in their early years but may become disadvantageous in their late years [107]. Mitochondrial haplogroups associated with lower oxygen consumption such as the phylogenetically related haplogroups U, K and J have been associated with protective effects against some of the common late-onset diseases and conditions. Sub-haplogroup N9 (a subclade of the macrohaplogroup N which is a common ancestor for haplogroups H, J, U, T and K) was shown to be associated with decreased risk for neurological decline after ischemic stroke in populations of Asian ethnic origin [108]. Sub-haplogroup N9a has also been found to protect its carriers from diabetes type 2 and metabolic syndrome [109] therefore, the protective effects against adverse outcomes after stroke may have been indirect. Mitochondrial haplogroups J, U, K and T and the unrelated haplogroup X were consistently seen more commonly in healthy individuals over 90 than in the healthy 'younger old' [95, 110, 111]. Carriership of haplogroup K (common in Western Europe and the British Islands) may protect against transient ischaemic attack and ischemic stroke [112]. In individuals with traumatic brain injury carriership of mitochondrial DNA of haplogroup K was associated with significantly better outcomes 6 months after the injury [113]. According to the cited study, the effects of carriership of haplogroup K were independent from the effects of carriership of *APOE4*. Generally, carriership of *APOE4* is associated with poorer outcomes after brain injury, but carriership

of mitochondrial DNA of haplogroup K apparently neutralised the effects of *APOE4* [113]. Haplogroups K and the closely related haplogroup U were demonstrated to have a protective effect against AD in European populations [114]. Individuals with mitochondrial genotypes of the haplogroups J, K, and T were at reduced risk of late-onset PD [115, confirmed in a larger study in 104]. Haplogroup J mitochondrial DNA is associated with lowest oxygen consumption and lowest efficiency of electron transport chain and, respectively, lowest ATP and ROS production of all common haplogroups [31, 96]. It may be speculated that this may result in lower levels of oxidative stress in mitochondria carrying DNA of haplogroup J than in mitochondria carrying DNA of other haplogroups. Nevertheless, the relationship between carriership of specific mitochondrial haplogroups and risk for neurodegenerative disease may not be always straightforward. Some authors have reported that, at least in some populations, haplogroup J was actually more common in AD patients than in healthy controls [116]. Other studies reported that the U and K haplogroup were associated with increased risk for AD [117]. In 2012, another study reported that carriership of haplogroup J was associated with increased risk for cognitive impairment (but not overt dementia), whereas individuals with haplogroup T had a nearly 2-fold increase in the risk for developing dementia [102]. Thus, the increased risk for neurodegenerative disease for carriers of haplogroup H was repeatedly confirmed whereas the expected protective effects of haplogroups with oxygen consumption and ROS production rates at the lower end of the scale were not readily apparent in all populations. Additional factors may modulate the effect of carriership of selected mitochondrial haplogroups on the risk for late-onset neurodegenerative disease. Among these factors prime candidates are biological gender and carrier status for *APOE4*, both known independent modulators of the risk for AD and PD. In 2001, Carrieri et al. demonstrated that haplogroups K and U were less common in Italian patients with AD that were also *APOE4* carriers than in AD patients with genotypes that did not contain the *APOE4* allele. The findings in the cited study were interpreted as partial compensation of the risk conferred by carriership of *APOE4* by carriership of haplogroups K and U [114]. Thus, the decrease of the risk conferred by haplogroups K and U may be significant only for carriers of *APOE4*. Later studies demonstrated that females carrying the U haplogroup had a significantly *decreased* risk whereas males with haplogroup U had a significantly *increased* risk of AD [118]. Considering that the overall risk for women for developing AD is generally higher than for men, mitochondrial haplogroup U may be one of the few factors that may reverse the male-to-female ratio of AD.

6. Don't throw away, repair it - capacity to repair genotoxic damage may be critically important for the survival of adult CNS neurons

Normal adult neurons employ specific mechanisms in order to extend their lifespan and preserve their functional capacity for years and decades [119]. They selectively downregulate nucleotide excision repair (NER) in the nontranscribed regions of the genome, focusing virtually all of its capacity onto the transcribed regions [119, 120]. Unrepaired damage may accumulate with time in the untranscribed regions of the genome, but since differentiated neurons are not expected to divide ever again, there is virtually no risk that damaged or altered DNA may be transmitted to neuronal progeny [120, 121]. Nontranscribed strands of transcribed genes are also repaired with priority in differentiated neurons (unlike most cells where transcribed strands of transcribed genes are repaired with the priority mechanism of transcription-dependent repair whereas the untranscribed strands are repaired by the much slower mechanism of global genomic repair) [120]. Both mechanisms work together to ensure the integrity of the functional parts of the nuclear genome of the adult neuron with minimal risks associated with accumulation of unrepaired damage. Repair of mitochondrial DNA has special importance for differentiated neurons, as they depend heavily on the ROS-generating mechanism of oxidative phosphorylation to provide chemical energy. Mitochondria have their own DNA repair profile, which may, for most cell types, be quite different from the repair profile of the nuclear genes. Mitochondria apparently have no use for NER [122]. Damage that is usually repaired by nucleotide excision (e.g. pyrimidine dimers) may be left unrepaired in mitochondrial DNA unless it could be repaired by some of the other repair mechanisms [123]. Thus, in differentiated neurons base excision repair (BER, the main mechanism normally used for repair of oxidised bases); mismatch repair (managing potential errors introduced during the synthetic phase of BER) and, possibly, homologous recombination are responsible for the maintenance of all nuclear DNA and the mitochondrial DNA, while NER (the most versatile repair mechanism, being capable of repairing almost any type of damage) is available only for some parts of the genome. As oxidation is the most common type of damage in neuronal DNA, the functionality of the cell machinery for repair of oxidised bases (BER) and of strand breaks (recombination) may be critically important for the normal functioning of neurons. To date, no monogenic human diseases associated with deficiency of BER have been described. It is believed that the phenotype that may potentially arise from inherited defects in genes coding for proteins of BER is very severe

and causes early death in utero. The role of dysfunctional BER (because of increased damage burden and/or because of decreased capacity for BER), nevertheless, has been implicated in the pathogenesis of a number of common late-onset diseases and conditions with multifactorial genesis. Recently, a consistent up-regulation in the expression of the genes *MSH2* (coding for a component of the system for recognition of mismatched bases) and *XRCC1* (coding for an accessory factor of ligase III, the main ligase of BER) and *ATM* (functioning in damage-related signalling and decision-making about the fate of damaged cells) was reported in patients with insulin resistant diabetes and coronary disease [124]. This was interpreted as an attempted compensation for the increased levels of genotoxic damage characteristic of diabetes type 2. Induced upregulation of the expression of key BER proteins may protect from excessive cell and tissue damage. In ischemically preconditioned rats, the levels of expression of XRCC1, LIG3 α (coding for ligase III) and PolB (DNA polymerase beta, one of the main polymerases of BER) were higher than in control (ischemia-naïve) rats and the rates of neuronal cell death after induction of brain ischemia were lower than in control rats [125]. This may, potentially, be used as a mechanism to limit tissue damage in conditions associated with increased levels of oxidative stress (vascular disease, diabetes, etc.)

Carriership of variant alleles of genes coding for major proteins of DNA repair by BER and mismatch repair has been associated with increased risk for common late-onset diseases. Allelic variants of the BER glycosylase *Neil1* conferring subtle enzyme deficiency has been shown to produce an obese, dyslipidemic and insulin resistant phenotype in mice resembling human metabolic syndrome [126]. Mitochondrial DNA of *Neil1*-mutant mice exhibited increased levels of DNA damage and deletions than wildtype controls. Mutations in human *NEIL1* gene are identifiable in 2-3 % of human patients with diabetes type 2 [127]. Insulin resistant diabetes has been suspected as a predisposing factor for cancer [128, 129] as well as late-onset dementia [130, 131]. The latter may be an effect related to the increased rates of vascular disease in individuals with unmanaged diabetes, but the authors of the cited studies reported decreased rates of clearance of amyloid beta peptide in individuals with high insulin levels. The activity of Ogg1 (8-oxoguanine glycosylase, the main enzymatic activity removing oxidised guanines from DNA) was revealed to be critically important in the repair of oxidative lesions after brain ischemia. *Ogg1*-null mice exhibited larger cortical infarcts after unilateral permanent middle cerebral artery occlusion (a model of stroke) and had significantly impaired recovery compared to control mice [132]. Carriership of polymorphic variants of *Ogg1* was shown to potentiate the expansion of the GAG repeat in the *Hdh* gene (the murine equivalent of

the human *HTT* gene) in mice modelling Huntington's disease [133]. Mutations in the murine *Msh2* gene were associated with increased risk for inter-generational expansion in the CGG repeat in a fragile X premutation mouse model [134]. More than 20 expansion diseases are currently known, most of which present with late-onset neurodegeneration. The most common expansion disease - Fragile X syndrome is not a neurodegenerative disorder, but carriers of premutation alleles of the CGG repeat in the human *FMRI* locus are at increased risk for a condition characterised by late-onset dementia and parkinsonism-like features (fragile X tremor/ataxia syndrome (FXTAS)), characterised by intention tremor, cognitive decline and generalised brain atrophy [135]. The polymorphic allele of the common Ser236Cys polymorphism in the human *OGG1* gene has been shown to be significantly associated with insulin resistance [136]. Carriers of the 326Cys allele were at twofold increased risk for sporadic motor neuron disease [137]. Variant alleles of polymorphisms in the genes coding for human apurinic/apyrimidinic endonuclease APE1 (the main nuclease hydrolysing the phosphodiester backbone 5'- from the abasic site generated by BER glycosylases) and the gene coding for XRCC1, the stabilising factor of ligase III (the primary ligase of BER) were identified as predisposing factors for sporadic Parkinson's disease [138]. Deficiency of DNA polymerase beta was reported to accelerate cognitive decline in transgenic mice modelling AD [139].

The importance of BER notwithstanding, subtle deficiencies of repair by NER may also affect the risk for neurological impairment. Repair of transcribed regions is vitally important for the maintenance of genomic integrity in adult neurons and inherited repair deficiencies affecting transcription-dependent NER usually present with severe, early-onset neurological impairment (xeroderma pigmentosum of complementation groups A, B and D, Cockayne syndrome). Repair of untranscribed genomic regions does not play significant role in the maintenance of genomic integrity in adult neurons, but may be crucial for tissues with rapid turnover (e.g. vascular endothelium), thereby modulating the risk for cerebrovascular disease. NER deficiency may play a role in the pathogenesis of vascular disease, stroke and post-stroke cognitive decline. Recently it was demonstrated that subtle deficiency of a major NER protein due to carriership of an insertion/deletion polymorphism in intron 11 allele of the human *XPC* gene (coding for one of the components of the complex that recognises damage in untranscribed DNA) may increase the risk for cerebrovascular disease and stroke [140].

DNA breaks secondary to oxidation are also a common type of damage in mitochondrial DNA. DNA breaks are generally the least tolerated type of DNA damage and their presence may directly activate programmed cell death pathways. Contrary to the long-established no-

recombination' rule for mitochondrial DNA, it is now believed that breaks in mitochondrial DNA are repaired by a recombination mechanism mediated by a RAD52-like single-strand binding protein [141]. The polymorphic allele of the Thr241Met polymorphism in the *XRCC3* gene (coding for a component of the XRCC2-XRCC3-RAD51 complex responsible for the branch migration and the resolution of the recombinant molecules in homologous recombination) is associated with increased risk for sporadic Parkinson's disease [138]. Deletions flanked by direct repeats (most likely resulting from non-reciprocal recombination) are commonly observed in mitochondrial DNA of aged cells [142, 143]. Considering the very high gene density of mitochondria, the age-related occurrence of mitochondrial deletions may contribute to the decline in the efficiency of oxidative phosphorylation and, respectively, to the age-dependent increase in the amount of ROS, thereby increasing the risk for occurrence of genotoxic damage. In 2006 it was demonstrated that the incidence of serious structural defects (mainly, deletions) in mitochondrial DNA increased with age, resulting in late-onset deficiencies in the electron transport chain that increased further the oxidative stress in aging neurons [91]. Several years later it was shown that large deletions in mitochondrial DNA affecting the cytochrome c complex (COX) were commonly found in the hippocampus of autopsied brains of patients with AD [92]. It was proposed that the underlying mechanism for COX deficiency was based on age-dependent accumulation of small deletions in the mitochondrial DNA that, eventually, obliterated the COX complex or, at least, made it severely dysfunctional. It is unlikely that even a normally functioning mechanism for repair of genotoxic damage would succeed in managing the augmented influx of ROS generated by mitochondria with dysfunctional electron transport chain. Thus, neuronal cell death could be expected to occur sooner rather than later in the context of genetic and/or age-dependent repair deficiency.

Identification and repair of genotoxic damage is only one aspect of the functioning of the cellular repair machinery. Assessment of the genomic integrity (presence of residual unrepaired damage and its potential reparability, proximity to the apoptotic threshold) is another, equally important component of individual repair capacity. Signalling for the presence of unrepaired damage may be relayed via several major proteins with damage-sensing and executive functions - BRCA, ATM, MSH2, MSH6 (all parts of the BRCA1-associated genome surveillance complex involved in the recognition of aberrant DNA structures) and the master regulator protein p53 [144-146]. Most of these genes are highly conserved and alterations in their DNA sequence or structure results in cancer-prone phenotypes with early (*ATM*) or late onset (*TP53*, *BRCA*, *MSH2* and *MSH6*). Nevertheless, relatively benign polymorphic changes have

been described in the genes coding for proteins functioning in the maintenance of genomic integrity. Single-nucleotide changes in *ATM* decrease the activity of the ATM protein, accelerating accumulation of DNA damage. Homozygous carriers of ATM mutations exhibit increased susceptibility to cancer and increased rates of apoptosis in specific cell populations with inherently high levels of DNA damage (thymus - due to physiological introduction of DNA breaks during T-cell receptor gene assembly, cerebellum - due to DNA damage inflicted by ROS generated by oxidative phosphorylation). Heterozygous carriership of *ATM* mutations is quite common (about 2 % in most populations and > 10 % in some) [147]. Heterozygote carriers of *ATM* mutations have been found to be at higher risk of death than non-carriers due to any disease-related causes and at any age between 20 and 79, but the risk was specifically increased (2-2.5-fold) for cancer and cardiovascular disease [148]. The age of death in carriers of mutant *ATM* alleles was 11 years younger than noncarriers for cardiovascular disease and 4 years younger for cancer. In transgenic *APOE*-expressing mice carriers of one mutant *ATM* allele exhibited significantly increased insulin resistance and accelerated vascular disease independently of carriership of *APOE* variant alleles [149]. Carriership of variant forms of *ATM* may indirectly modulate the risk for dementia in later life by increasing the risk for vascular disease and stroke. No polymorphism in the *ATM* gene has so far been shown to influence the risk for dementia in late life or any of the factors that may increase the risk for dementia (vascular disease, insulin resistance, etc.), although the rs4585 T/G polymorphism was shown to modulate the glycemic response to metformin (thereby indirectly influencing the level of oxidative damage in diabetes) [150].

p53 is a master transcription regulator and controller of cell fate [145, 151]. Several polymorphisms have been described in the *TP53* gene, three of which (Pro72Arg in exon 4, Pro47Ser - also in exon 4 and 16-bp duplication in intron 3) [152-154] are quite common, although their prevalence may be different in different populations. Both polymorphisms in exon 4 modulate the capacity for induction of cell cycle arrest or induction of apoptosis in the presence of DNA damage [155, 156]. No significant associations with susceptibility for human disease have been identified for the *TP53* Pro47Ser polymorphism [157]. The duplication allele of the 16 bp duplication polymorphism in intron 3 is associated with a subtle deficiency in virtually all of p53 functions - damage-associated cell cycle arrest, DNA repair and induction of apoptosis conferring slightly increased risk for common cancers [154, 158]. It is possible that it may play a role in the susceptibility to late-onset disease, but since the overall number of studies in the field of individual repair capacity is still limited, no definitive association has been elicited yet. The Pro72Arg polymorphism has been

extensively studied, revealing associations with the risk for various human diseases and conditions and their potential complications; as well as with human longevity. The two alleles of the Pro72Arg polymorphism confer essentially the same DNA-binding properties, and the resultant variant proteins are conformationally indistinguishable from each other [152, 155]. The *TP53* 72Pro and 72Arg variant are, nevertheless, significantly different with regard to their potential downstream targets. The 72Pro variant is a stronger inducer of transcription of proteins related to cell cycle arrest and DNA repair in damaged cells than 72Arg whereas the latter induces apoptosis more effectively than 72Pro [155]. Carriership of the arginine allele of the *TP53* polymorphism is linked to increased ischemic damage to myocardium after cardiac ischemia (in an age-dependent manner, with patients over 60 being significantly more sensitive to its effect than patients in their thirties) [159] and higher risk of recurrence of vascular disease after coronary bypass [160]. Arg72 carriers may have poorer prognosis after ischemic stroke, probably because of increased risk that cells damaged by the excessive production of ROS in the penumbra of the ischemic focus and vulnerable neurons elsewhere in the brain may die in the post-stroke period [161]. At present, there is little published evidence about the role of Pro72Arg polymorphism in the risk for late-onset dementia, except for data about the potential role of the polymorphism in the establishment of risk for cerebrovascular disease and vascular cognitive decline [162, 163]. To date, there have been several studies attempting to find an association between the risk for development of sporadic late-onset AD and carriership of allelic variants of *TP53*, but they have not succeeded in eliciting a link [164, 165]. It has been known, nevertheless, that the 72Pro variant and the 72Pro/Pro homozygous genotype is overrepresented in individuals that have remained healthy well into their old age ('successful agers') and in elderly individuals that have survived cancer [166, 167]. Notably, the cited studies have been carried out in populations where the ancestral 72Pro allele is less common than the 72Arg allele, thus minimising the risk for sampling bias. It is possible that the increased propensity for apoptosis conferred by the 72Arg allele may increase the risk for degenerative disease whereas the 72Pro allele may increase the risk for cancer in advanced age. If carriers of 72Arg (and, possibly, other pro-apoptotic factors) possess an efficient system for identification and repair of genotoxic damage, the threshold of damage that sends damaged cells along the apoptotic pathway may be reached very late in life. In tissues with slow turnover, as is neural tissue, this may mean that the majority of the neurons may well live until the organism lives. In tissues where cell turnover is more rapid, the 'pro-apoptotic tendency' conferred by 72Arg allele/s may increase the risk for

degenerative disease. In carriers of alleles conferring lower-than average capacity to repair genotoxic damage and increased capacity to eliminate damaged cells, many tissues would suffer with advanced age, but for most of these replacement of damaged cells would be readily available (at least until very old age), whereas neuronal tissue may suffer early demise, as it is difficult to replace. Thus, individuals with such genetic backgrounds may be specifically predisposed to late-onset neurodegeneration and, potentially, vascular disease. In individuals carrying the 'pro-repair' 72Pro allele there may be increased risk for cancer in advanced age, although the individual risk and the potential outcomes would depend on the genetic background conferring normal or lower-than-average capacity to repair genotoxic damage. In the case of normal repair capacity plus 'pro-repair' genotype there may be risk that cancer may occur at later age. At the same time, there may be increased likelihood that genotoxic anticancer treatments may be successful, as cancer cells with decreased repair capacity are more prone to die or arrest their rapid division under conditions of increased genotoxic stress inflicted by genotoxic therapy [32, 168]. Thus, individuals with normal repair capacity plus 'pro-repair' genotype may live well into old age as cancer survivors.

The effects of carriership of genetic variants conferring subtle variance in repair capacity and/or maintenance of genomic integrity may be modulated by concomitant inheritance of mitochondrial variants conferring increased (haplogroup H) or decreased (haplogroups K, T, U and J) production of ROS and *APOE* variant alleles. One may expect that the genotype of the 'successful ager' may be comprised of *APOE3/E3* (or *E2/E3*); mitochondrial DNA of haplogroup K, T, U or J, normal capacity to repair genotoxic damage (that usually means carriership of ancestral (non-variant) alleles of known polymorphisms in genes coding for proteins of DNA damage identification and repair), at least one *TP53* 72Pro allele and absence of heterozygous *ATM* mutations. Certainly, environmental factors, lifestyle and habits would also play a role.

Further studies in the field of individual repair capacity and maintenance of genomic integrity are strongly advocated in order to elucidate the mechanisms of neuronal and vascular dysfunction in later age and to identify potential targets for therapeutic intervention. These include population studies of the potential effects of carriership of allelic variants of genes coding for proteins of repair and maintenance of genomic integrity on the risk for late-onset disease [162, 168, 169] as well as personalised studies of capacity to repair genotoxic damage and/or the capacity for self-renewal in individually collected cultured cells [170-173].

Until research into the molecular bases of common late-onset disease has progressed sufficiently to ensure

reliability of prognostication of the risk for development of common late-onset diseases and their potential complications, there is not much that could be done about genetic predisposition to late-onset disease. Scientific knowledge about the interlinked mechanisms that determine the outcomes of the same disease in different individuals is still limited. The risk assessments based on family history and genetic testing are not always reliable. Etiologic and/or efficient symptomatic therapies are still unavailable for many of the common late-onset diseases, including dementia in late life. One could, however, manage the controllable factors of lifestyle (maintenance of optimal body weight for age and sex, moderate physical activity, arterial pressure 120/80 or lower, blood glucose and cholesterol within reference ranges, use of preventive anticoagulation, diet rich in natural antioxidants and free radical scavengers) so as not to increase further the risk for late-onset disease. It is possible that individuals at increased risk for late-onset dementia (e.g. individuals diagnosed with mild cognitive decline) may benefit from long-term antioxidant therapies (in addition to lifestyle alterations) in order to decrease the risk for cerebrovascular disease due to amyloid or atherosclerotic neuropathology.

Conclusions

Three major factors are likely to play significant roles in the constitution of the genetic risk for sporadic late-onset dementia - 1) carriership of *APOE* variant alleles (specifically, the E4 allele); 2) mitochondrial DNA haplogroup and 3) genetic capacity to identify and repair oxidative damage and make decisions about the fate of damaged cells (individual repair capacity). The individual contributions of these genetic factors to the risk for late-onset disease may be subtle, but when combined in the same genotype, they may significantly increase in an age-dependent manner the individual risk for Alzheimer's dementia and vascular dementia in susceptible individuals even in the absence of positive family history. More research into the bases of common late-onset disease may be needed in order to establish reliable estimates for the genetic risk of late-onset dementia. Meanwhile, a combined therapeutic approach comprised of minimisation of the environmental factors increasing the risk for vascular disease and antioxidant therapies may be practical in order to decrease the risk for late-onset dementia.

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